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EFFECTS OF SOMATOTROPIN INJECTION AND FEEDING FREQUENCY ON PLASMA HORMONE CONCENTRATIONS, RUMEN PARAMETERS, MILK YIELD AND MILK COMPOSITION IN HOLSTEIN COWS

by

NEIL JOHN FRENCH

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

Animal Nutrition

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1988

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The undersigned certfy that they have read, and recomended to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EFFECT OF SOMATOTROPIN INJECTION AND FEEDING FREQUENCY ON PLASMA HORMONE CONCENTRATIONS, RUMEN PARAMETERS, MILK YIELD AND MILK COMPOSITION IN HOLSTEIN COWS submitted by NEIL JOHN FRENCH in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

.....

Supervisor

.....

.....

Date March 18, 1988

Dedication

To my wife Alicia

and son Andrew

Abstract

Two experiments were conducted to examine the effects of feeding frequency on milk yield, milk composition, rumen parameters, and plasma insulin concentrations of Holstein cows. Feeding frequency had no effect on milk yield, milk protein, or milk lactose in either experiment. Increased feeding frequency increased ($P<0.05$) milk fat from 2.21 to 2.60% in experiment 1 but had no effect ($P>0.05$) on milk fat in experiment 2. Increased feeding frequency increased average rumen pH and acetate/propionate ratio in experiment 1, as well as reducing postprandial fluctuations of both rumen parameters. Feeding frequency had no affect on rumen parameters in experiment 2. Increased feeding frequency tended ($p<0.1$) to reduce plasma insulin in experiment 1 but had no effect in experiment 2. These results support previous work which indicate that the effects of increased feeding frequency are more pronounced when milk fat depression is most severe.

In a third experiment, effects of bovine somatotropin (BST) injection and feeding frequency on milk yield, milk composition, and plasma hormones were examined. Feeding frequency had no effects on any parameters measured, nor was there an interaction of BST with feeding frequency. Somatotropin injection increased ($P<0.05$) milk production by 4.4 kg/day per cow. Concentration of fat, protein, lactose and fatty acid composition of the milk were unaffected by BST injection. Fatty acid composition of the

milk was also unaffected by injection. Feed intake and body weight were unaffected by treatment. Average plasma BST cncentrations were elevated 3 fold and peak concentrations were increased 7 fold by BST injection. Plasma BST concentration remained 1.5 times control values at 48 hours post injection. Plasma insulin and plasma glucose concentrations were unaffected by treatment. These results indicate that BST injection has potential to increase milk production under a variety of feeding conditions.

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I. INTRODUCTION

In dairy cattle considerable research effort has been focussed on increasing milk yield and the efficiency of milk production. Through better knowledge of the dairy cows nutrient requirements, substantial improvements in milk production have been acheived. However, there are large differences between animals in their ability to produce milk. While genetic selection has resulted in animals of superior ability to produce milk, our knowledge of how biological changes contribute to increased milk production is lacking. In animals fed equal amounts of an identical diet, differences in digestion or absorption of nutrients are small (3). However, there are major differences between animals in the partitioning of nutrients between body tissue stores and milk production. Some animals decrease body reserves to support high levels of milk production while others may actually increase body reserves while producing low levels of milk production (3). An understanding of the factors controlling nutrient partitioning may allow for the opportunity of increasing milk production.

Some researchers have demonstrated a relationship between plasma hormone concentrations and nutrient partitioning (1,6,11,35). In dairy cows, plasma somatotropin (BST) concentrations and plasma insulin concentrations are important factors in nutrient partitioning (1,6,11,35). In early lactation, when milk production is highest and animals tend to

be in a negative energy balance, plasma BST concentrations are highest, while plasma insulin concentrations are at their lowest level (1,11,17,36). As lactation proceeds and milk production declines, BST concentrations decline and plasma insulin concentrations increase (1,11,17,36). When receiving equal quantities of an identical diet, high milk producing cows had higher plasma BST concentrations and lower plasma insulin concentrations than their low milk producing counterparts (17). However, energy balance differed between the high and low milk producing animals and both BST and insulin are known to be influenced by energy balance of the animal (1). Thus, it is a matter of debate whether these hormones cause differences in nutrient partitioning or whether changes in energy balance, due to a shift in nutrient partitioning, results in changes in plasma hormone concentrations. Experiments showing a decline in milk production following insulin injection (34), and increases in milk production following BST injection (4,8,24) seem to support the theory that alterations in nutrient partitioning are a result of changes in the ratio and concentration of key hormones such as BST and insulin.

In high producing dairy cows glucose availability has been implicated as being limiting for milk lactose formation (1), therefore, hormonal control of glucose metabolism may be important in regulating milk yield. Since glucose absorption in ruminants is minimal, glucose needs are met primarily by gluconeogenesis. At the onset of lactation gluconeogenesis has

been shown to increase (5) as a result of increased plasma BST and decreased plasma insulin concentrations. While these changes affect gluconeogenesis, their most important effect may be on glucose partitioning. It has been shown that at 30 days prepartum, up to 34% of glucose is oxidized to CO_2, while at the onset of lactation this may drop to 8% (1). This could be attributed to changing hormone levels, as insulin promotes uptake of glucose by adipose tissue while BST may act to block this action (1). Thus, a decrease in insulin and (or) an increase in plasma BST concentrations should reduce glucose uptake by adipose tissue and hence increase glucose availablity to the mammary gland. This theory is supported by the fact that insulin decreases milk yield (34) while BST increases milk yield (4,8,24). Also, BST may be diabetogenic in vivo (20) and may actually increase circulating glucose concentration in low producing cows (17). Insulin and BST may also have important effects on adipose tissue. In early lactation, cows are usually in a negative energy balance. This deficit can be energetically equivalent to as much as 50 Kg of lipid (1). As previously discussed, insulin promotes uptake of glucose and BST antagonizes this effect. It has also been shown that insulin is needed for incorporation of acetate by adipose tissue and again, this is antagonized by BST (37). Thus, increased BST and(or) decreased plasma insulin concentration reduce fatty acid synthesis in adipose tissue making more nutrients available to the mammary gland. Insulin and BST may also indirectly affect

lipolysis. Insulin has been shown to antagonize the effects of glucagon and catecholamines, both of which increase lipolysis (19). Somatotropin on the other hand has been shown to enhance the effects of catecholamines on free fatty acid release in vivo (20). This is supported by in vitro results where adipose tissue taken from animals in early lactation, when plasma BST concentrations are high, was less responsive to catecholamines than adipose tissue taken from animals in later lactation when plasma BST concentrations are lower (26). Whether BST has an intrinsic lipolytic effect is still being debated. Some workers believe it does while others suggest its effect is indirect (20) with lypolysis being increased only as a result of increased demand by the mammary gland. Regardless of the mechanism, injections of BST have been shown, under some conditions, to increase plasma free fatty acid concentrations.

In addition to its effects on glucose and fatty acid partitioning, BST may act indirectly on the mammary gland to increase milk synthesis. While BST does not have a direct effect on the mammary gland there is increasing evidence that BST may act through insulin-like growth factors (IGF-1). Somatotropin injection has been shown to increase IGF-1 concentrations in plasma (13,32). It has also been demonstrated that IGF-1 may enhance synthesis of milk components by the mammary gland in vitro (9). This theory of IGF-1 action is further supported by the fact that infusion of nutrients does not increase milk production to the same extent as BST injection

(30). Further support is provided by increased mammary blood flow following BST injection in goats (18) and dairy cows (12). In most tissues, blood flow increases through several autoregulatory methods in response to increased metabolism (16).

It is clear from the foregoing that BST and insulin are important factors in the control of nutrient partitioning and mammary metabolism. One possible method of increasing BST levels may be by increasing the energy deficit (6,14); however, this is not an effective way to increase milk production. It has also been shown that increased amino acid supply to the small intestine may raise plasma BST levels (27). However, this is expensive and conflicting results have been obtained (30). Another method of raising plasma BST concentrations has been by infusion of arginine (10), however, this is not practical under commercial conditions. With current genetic engineering technology providing an ample low cost supply of BST, the simplest method of elevating plasma BST concentrations is by injections (4,20).

While the goal of increased milk production requires increased BST concentrations the opposite is true for insulin. Insulin secretion in ruminants appears to be controlled by volatile fatty acid (VFA) concentrations in blood (35), with propionate being more potent than acetate in stimulating insulin secretion. When a diet high in concentrates is fed to ruminants there is increased VFA production, rumen pH declines and there is a shift in fermentation with increased propionate production

(22,23,28). Since propionate stimulates insulin secretion, this could explain the high serum insulin concentrations and milk fat depression observed in cows fed high concentrate diets (21,22,38). A possible solution is to decrease the concentrate portion of the diet, however this may result in decreased milk production due to limitation in energy intake. An alternative approach would be to feed a high concentrate diet in smaller portions at more frequent intervals. This has been shown to result in increased rumen pH and decreased propionate production (23) which may result in decreased postprandial insulin secretion and an increased milk fat percent (19). Thus, the most practical method of manipulating plasma insulin concentrations may be by increased feeding frequency.

The objectives of this research were to determine the influence of BST injection on plasma BST and insulin concentration, and on milk yield and milk composition. Effects of feeding frequency on rumen parameters, plasma insulin concentrations and on milk yield and milk composition were also examined. Finally, the combined effects of BST injection and feeding frequency on plasma BST concentrations, plasma insulin concentrations, milk yield and milk composition were investigated.

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II. EFFECTS OF FEEDING FREQUENCY ON RUMEN PARAMETERS, PLASMA INSULIN, MILK YIELD, AND MILK COMPOSITION IN HOLSTEIN COWS

A. INTRODUCTION

Developments in automated feeding equipment have made it possible to feed concentrates to dairy cattle in small portions at frequent intervals. Promotional literature for these automated systems suggest that increased feeding frequency should result in higher milk production and increased milk fat percent. However, evidence cited by this literature is often without statistical controls and, therefore, of questionable value. Controlled research on this subject has produced mixed results. A recent review on the effects of increased feeding frequency (10) showed milk yield to be increased in four experiments, unchanged in 24 and decreased in seven studies. The overall increase in milk yield associated with increased feeding frequency was found to be 2.7%. Milk protein and lactose concentration were unaffected by feeding frequency. Of parameters measured, milk fat concentration showed the largest response to increased feeding frequency with an overall increase of 7.3%. However, the effect of increased feeding frequency was variable with seven experiments showing increased milk fat concentration while 27 showed no response. Differences in initial milk fat concentration may explain the conflicting results associated with increased feeding frequency (10). In

general, where milk fat percent is low with twice daily feeding, increased feeding frequency tends to have a positive influence on milk fat concentration.

The form and composition of the diet is a major factor influencing milk fat concentration. High concentrate, low roughage diets appear to cause milk fat depression (16, 18). This condition tends to be aggravated if either the forage or the concentrate is finely ground or pelleted (18). Under these conditions, propionate production tends to increase resulting in a lower acetate:propionate ratio with a concurrent decrease in rumen pH (17). Increased feeding frequency may result in a higher and more stable rumen pH and acetate:propionate ratio which in turn may cause milk fat concentration to increase (12, 25).

Feeding frequency may also influence milk fat percent through an effect on insulin secretion. Insulin promotes storage of glucose and acetate by body tissues, especially adipose tissue, thereby reducing their availability for milk fat synthesis by the mammary gland (3). Insulin secretion in ruminants appears to be controlled by VFA concentrations in blood (23) with propionate being more potent than acetate in stimulating insulin secretion. In animals fed high concentrate diets there is increased ruminal propionate production (2) which may result in elevated plasma insulin concentration, especially postprandially (12,14, 28). Increasing feeding frequency may help counteract the elevated ruminal propionate production and plasma insulin concentration on high concentrate diets thereby increasing milk fat percent (12).

The objectives of this study were to examine effects of feeding frequency on ruminal pH, VFA concentrations, plasma insulin concentration, milk yield and milk composition in Holstein cows fed a 60% pelleted concentrate, 40% chopped alfalfa hay diet.

B. MATERIALS AND METHODS

Animals, Diet, and Sampling Procedure:Experiment 1

Four ruminally cannulated Holstein cows were randomly assigned to one of two treatments in a crossover design. In treatment one (12X) a pelleted concentrate was fed in 12 equal portions at 2 h intervals using an automatic feeding device. In treatment two (2X) the pelleted concentrate was fed in two equal portions at 0500 h and 1500 h immediately following milking. Chopped alfalfa hay was fed to all animals in two equal portions at 0800 h and 1600 h. Diets (Table 1) contained 16% CP and were provided at a constant intake for both treatments at 20.7 kg DM/d. Treatment periods were 14 d with milk yield and milk composition recorded on d 11 to 13. Rumen and blood samples were collected hourly for 24 h beginning on d 14 at 0600 h. Rumen samples were collected using a syringe attached to a tube inserted through the rumen cannula, pH was determined immediately and samples were stored at -20°C. Blood samples (20ml) were collected via indwelling jugular catheters which were inserted on d 13. Blood was transferred to heparinized (12 I.U./ml of blood) glass tubes and placed on ice for up to 1 h until centrifuged for 15 min at 3000 rpm using a Sorvall GCL-2B

centrifuge. Plasma was transferred to plastic vials and frozen at -20°C. At the end of the sampling period animals were changed to alternate treatments and the procedure was repeated.

Animals, Diets and Sampling Procedure:Experiment 2

Six Holstein cows were used in a 3 X 3 Latin square design. Treatments were 12X and 2X daily feeding, as in experiment 1, plus a third treatment consisting of a total mixed ration (TMR) fed in 12 equal portions at 2 h intervals. Diet composition was the same as in experiment 1. Intake was kept constant at 20.7 kg DM/d. Treatment periods and milk sampling were as outlined for experiment 1. As animals in experiment 2 were not fitted with rumen cannulae, rumen fluid was collected by a stomach tube on an hourly basis from 0600 to 1100 h on d 14. Blood samples were taken on d 13 to avoid possible changes in plasma insulin due to stress associated with stomach tubing. Animals fed concentrate 2X daily had consumed their morning feeding of concentrate before rumen sampling began.

Analytical Procedure.

Milk was analyzed for fat, protein, and lactose using infrared analysis[1]. Rumen samples were thawed overnight at 4°C, strained and centrifuged. To 5 ml of the supernatant was added 1 ml of 25% meta-phosphoric acid and 1 ml of a caproic acid solution as an internal standard. Concentration of VFA's was determined by gas chromatography[2] using a Varian Model 3700 with flame ionization detector and a fused silica capillary

---

[1]Milkoscan 104; Manufactured by Foss Electric, Denmark.

[2]Supelco Inc., Bulletin 749B fast separation of VFA *C2-C5 Copywright 1975, Supelco Inc., Bellefonte, pennsylvania 16823.

column (30 cm long; .25 mm i.d.) coated with OV351[3]. The column was operated at 130°C internal temperature programmed at 10°C/min to 185°C. Nitrogen was used as a carrier gas at a flow rate of 1 ml/min. Hydrogen and air flow rates were 30 and 300 ml/min, respectively. Peak areas were measured using a Hewlett Packard 3353 data system.

Plasma insulin concentrations were determined by double antibody radioimmunoassay. A 100 µl plasma sample, or standard, was preincubated with guinea pig antiserum to bovine insulin[4] (200 µl, final dilution 1:165,000) for 24 h at 4°C in 12 X 75 mm glass tubes. The following day ^{125}I-labelled insulin (approximately 20,000 CPM in 200 µl) was added and the mixture was allowed to incubate for 48 h at 4°C. After incubation, bound hormone was precipitated using 100 µl goat antiserum to guinea pig gamma globulin[4] (final diulution 1:210) and 100 µl normal guinea pig serum[4] (final dilution 1:2100 and a 24 h incubation at 4°C). Tubes were centrifuged at 3000 rpm for 30 min at 4°C, supernatant was aspirated and the precipitate (bound hormone) was counted using a Beckman 8400 solid scintillation counter. Insulin, for standards and iodination was obtained from Sigma[5]. Iodination was carried out using the procedure of Greenwood et al. (11). The assay was tested for linearity, recovery, and parallelism. All samples were completed in one assay to remove inter-assay variation. Intra-assay variation was 10.5%.

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[3]Capillary column from G. and W. Scientific, Inc. 3871 Security Park Drive, Rancho Cordova, Ca. 95670, USA.

[4]Antibodies and normal guinea pig serum were purchased from Miles Laboratories, Inc. Elkhard, Indiana, 46515, USA.

[5]Sigma Chemical Co.; P.O. Box 14508, St Louis, Mo., 63178, USA.

C. RESULTS AND DISCUSSION

Milk Parameters

Results of effects of feeding frequency on milk yield and milk composition for experiments 1 and 2, respectively are in Tables 2 and 3. Feeding frequency had no ($P>.05$) effect on milk yield. This supports results in a recent review (10), where the average response to increased feeding was a nonsignificant increase of 2.7%. Milk yield was increased in only 4 of the 29 experiments reviewed and actually declined in 1 experiment suggesting that effects of feeding frequency on milk yield are small or nonexistant. Frequency of feeding also had no ($P>.05$) influence on milk protein or lactose which is in agreement with (10). In the present studies, the only parameter to show a response to increased feeding frequency was milk fat percent. In experiment 1, where milk fat percent was lowest, milk fat percent increased with increased feeding frequency while in experiment 2 where milk fat depression was less severe there was no response to feeding frequency. In reviewing the literature Gibsons (10) observed a similar trend.

Rumen Parameters.

Tables 4 and 5 show the average molar percent of VFA's, total VFA concentrations, acetate:propionate ratio, and rumen pH for experiments 1 and 2 respectively. In experiment 1 the proportion of acetate increased with 12X feeding while the proportion of propionate was decreased. As a result the acetate:propionate ratio was 38% higher with 12X feeding than

with 2X feeding. In experiment 1, rumen pH was also higher with 12X daily feeding. While mean acetate:propionate ratio and rumen pH were increased with increased feeding frequency, an even greater diurnal effect was observed (Figures 1 and 2). With 2X feeding acetate:propionate ratio declined after grain feeding, reaching a minimum at about 3 to 4 h post-feeding and remained low for 8 to 9 h post-feeding. The magnitude of postprandial decline in these parameters was reduced with 12X daily feeding. Rumen pH showed a similar pattern with animals fed twice daily having a large decrease in post-prandial pH which persisted for several hours before returning to prefeeding values. Again, the extent of variation was reduced with increased feeding frequency. In experiment 2 there were no differences between treatment groups in average or postprandial VFA concetrations, acetate:propionate ratio, or rumen pH.

Effects of increased feeding frequency on rumen fermentation patterns observed in experiment 1, and by others (15,17,19) may partially explain the observed change in milk fat percent. When large amounts of soluble carbohydrates are fed there is a rapid increase in rate of microbial fermentation and a decrease in rumen pH (Figure 2) and (17). Reduced rumen pH may influence milk fat percent through an effect on intraruminal vitamin B_{12} production which declines with rumen pH (9). Vitamin B_{12} is required for metabolism of propionate and if reduced there may be an accumulation of methylmalonate, a product of propionate metabolism, which in turn may interfere with milk fat formation (6,7,9). This would be further

complicated by increased propionate production. However, other work (8) has shown that methylmalonate concentration in plasma was not elevated on fat depressing diets, and that methylmalonate had no effect on mammary gland lipogenisis in vitro. Low rumen pH also tends to cause a shift from cellulolytic to amylolytic bacteria which may result in increased propionate production (Table 4) and (1,2,13,17), reduced acetate:propionate ratio. Propionate may have a direct effect on adipose tissue by stimulating lipogenesis and reducing lipolysis, thereby reducing availability of free fatty acids for milk fat synthesis by the mammary gland (20). Increased feeding frequency has previously been shown to increase the acetate:propionate ratio (17) and similar results were observed in experiment 1 (Table 4). This could explain a lack of response of milk fat percent to feeding frequency in experiment 2 as the acetate:propionate ratio was similar for all treatment groups. It is not clear why the acetate:propionate ratio was unchanged in experiment 2 as the diet was identical to that fed in experiment 1. However animals in experiment 2 had higher body weights (615 kg vs 540 kg) and because of this they may have been able to metabolize rumen products at a greater rate than animals in experiment 1, thus reducing their response to feeding frequency.

Plasma Insulin.

Increased feeding frequency tended ($P<.1$) to decrease both average and peak plasma insulin concentrations in experiment 1 (Table 4). Diurnal changes in insulin concentration were not

observed. Average and peak insulin concentrations in experiment 2 were unaffected by treatment (Table 5). These results differ from a previous report (12) where increased feeding frequency of a high concentrate diet decreased plasma insulin especially postprandially. Increased VFA production, especially that of propionate, in animals fed a high concentrate diet has been shown to increase plasma insulin concentration (23,26,27). Insulin affects adipose tissue by increasing lipogenesis and decreasing lipolisis (3,4,5, 21), thereby, reducing the availablity of free fatty acids for milk fat synthesis. Feeding large portions of concentrates in one or two feedings may cause a large postprandial increase in plasma insulin concentration and this has been implicated as a causitive factor in low milk fat syndrome (12). However, injections of insulin has resulted in decreased milk yield while milk fat yield was not signifigantly affected (24), hence milk fat percent actually increased. Insulin promotes storage of glucose, thereby, reducing the amount available for milk lactose production. Since lactose is an osmoregulator of milk volume, a decrease in glucose availability resulting from insulin injection may have reduced milk volume. These results of insulin injection do not support the theory that increased postprandial insulin caused depressed milk fat. However, responses to a pharmalogical dose of insulin may differ from those of physiological concentration. Even so high postprandial insulin should reduce milk volume as well as availability of free fatty acids for milk fat synthesis suggesting that it is not the causative factor in milk fat depression.

D. CONCLUSION

In two studies examining the effects of feeding frequency there was no effect on milk yield. However in experiment 1, where milk fat percent was lowest, increased feeding frequency did increase milk fat percent. Milk fat percent was unchanged in experiment 2. Effect of feeding frequency is maximal when animals are fed a rapidly fermented diet characterized by reduced rumen pH and increased propionate production. The causative factor in milk fat depression may be the direct action of increased propionate on adipose tissue, or the indirect action of an insulin induced reduction in fatty acid availability to the mammary gland.

Table II-1. Diet Composition, % as fed.

Chopped alfalfa hay	40.0
Barley	53.8
Molasses	2.2
Canola Meal	1.5
Calcium Carbonate	.8
Dicalcium Phosphate	1.1
Salt	.5
vitamin mix[1]	.2

[1]5000 I.U. vitamin A, 1280 I.U. vitamin D, and 5 I.U. vitamin E /kg

Table II-2. Effect of feeding frequency on milk yield and milk composition, experiment 1.

Feeding frequency	2X	12X	SEM
Milk yield, kg/d	29.8	30.5	1.26
Milk fat, %	2.21[a]	2.60[b]	.04
Fat yield, kg/d	.66	.80	.04
Protein, %	2.84	2.84	.07
Lactose, %	4.67	4.67	.09

2X, cows fed diet in two equal portions at 500 and1500 h.
12X, cows fed in twelve equal portions at 2 h intervals.
Means with with different superscripts differ,($P<.05$).

Table II-3. Effect of feeding frequency on milk yield and milk composition, experiment 2.

Feeding frequency	2X	12X	TMR	SEM
Milk yield, kg/d	36.5	37.3	33.5	3.4
Milk fat, %	2.97	3.03	3.34	.15
Fat yield, kg/d	1.08	1.13	1.12	.11
Protein, %	3.05	3.12	3.11	.11
Lactose, %	4.97	5.08	5.04	.13

2X, cows fed diet in two equal portions at 0500 and 1500 h.
12X, cows fed in twelve equal portions at 2 h intervals.
TMR, total mixed ration fed in 12 equal portions at 2 h intervals

Table II-4. Effect of feeding frequency on rumen parameters and plasma insulin concentrations, experiment 1.

Feeding frequency	2X	12X	SEM
Total VFA, mmol/L	104.6	110.6	19.1
Acetate, %	54.5	58.8	10.6
Propionate, %	34.8	28.4	7.5
Isobutyrate, %	.6	.8	.3
Butyrate, %	8.1	9.2	1.8
Isocalerate, %	.8	.9	.3
Valerate, %	2.3	1.9	.3
Acetate:propionate	1.6	2.2	.5
pH	5.7	6.2	1.4
Mean insulin, ng/ml	1.54	1.04	.8
Peak insulin, ng/ml	1.88	1.07	.9

2X, cows fed diet in two equal portions at 0500 and 1500 h.
12X, cows fed in twelve equal portions at 2 h intervals.
TMR, total mixed ration fed in 12 equal portions at 2 h intervals

Table II-5. Effect of feeding frequency on rumen parameters and plasma insulin concentrations, experiment 2.

Feeding frequency	2X	12X	7MR	SEM
Total VFA, mmol/L	87.2	79.2	71.0	20.3
Acetate, %	58.7	60.3	60.0	12.3
Propionate, %	27.4	27.4	27.4	7.4
Isobutyrate, %	.5	.6	.6	.3
Butyrate, %	9.2	9.1	9.0	2.1
Isovalerate, %	.9	1.0	1.9	.3
Valerate, %	1.7	1.6	1.7	.4
Acetate:propionate	2.1	2.3	2.2	.5
pH	6.2	6.4	6.3	1.4
Mean insulin, ng/ml	.91	.83	.87	.51
Peak insulin, ng/ml	1.11	.92	.89	.62

2X, cows fed diet in two equal portions at 0500 and 1500 h.
12X, cows fed in twelve equal portions at 2 h intervals.
TMR, total mixed ration fed in 12 equal portions at 2 h intervals

Fig. II-1. Effect of feeding frequency on rumen pH; 2-times daily feeding (open square, broken line) and 12-times daily feeding (closed square, solid line)

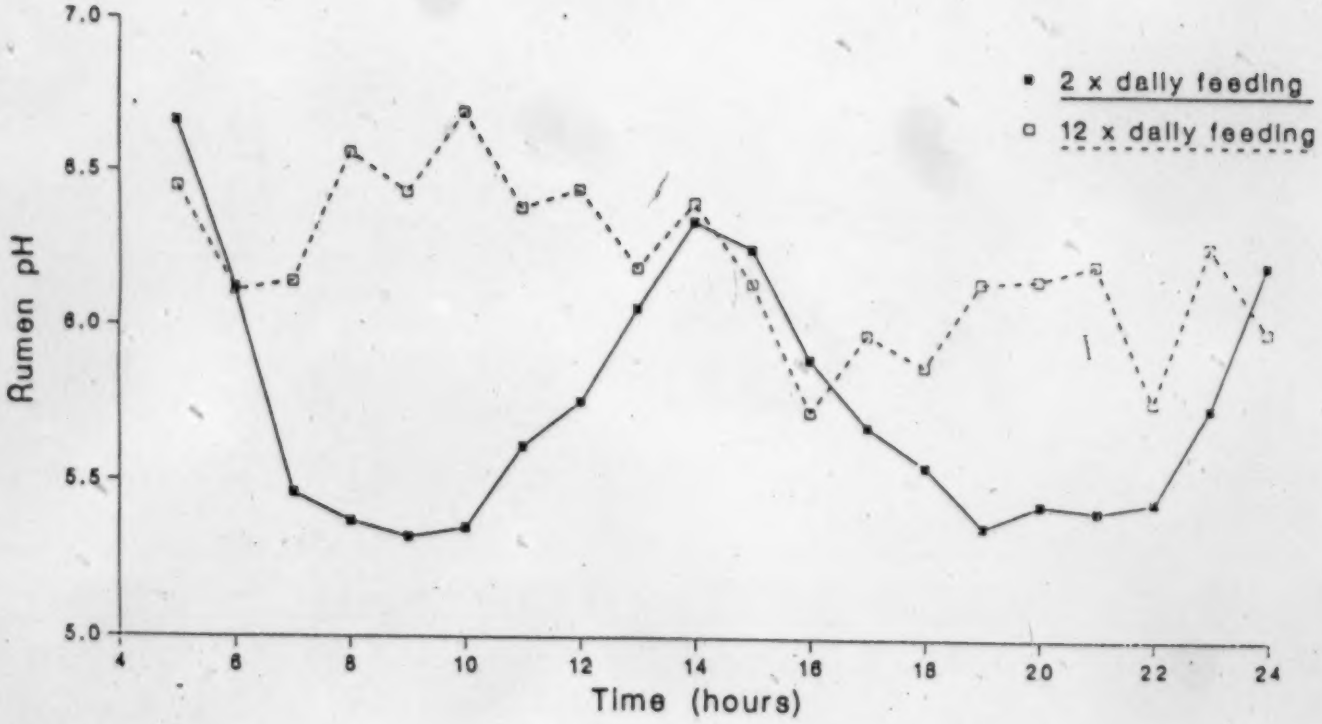
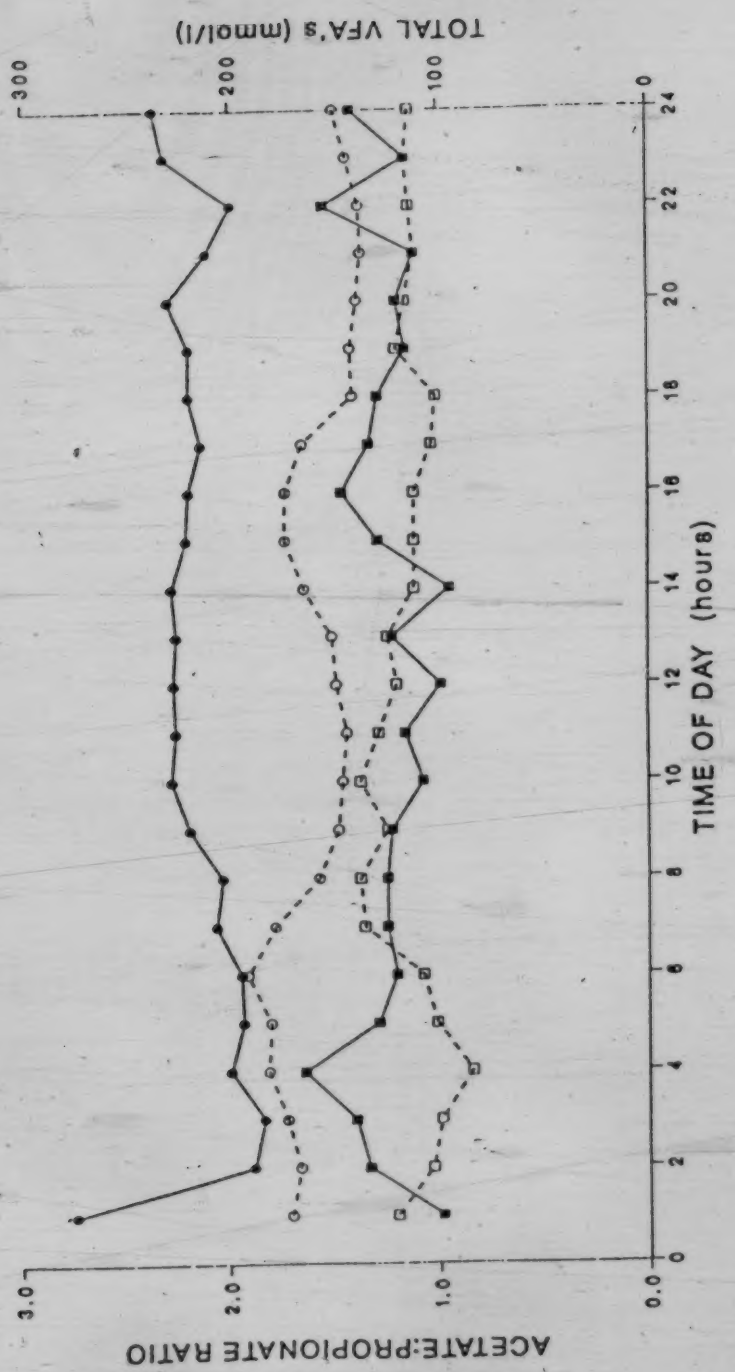

7.0
6.5
6.0
5.5
5.0
Rumen pH
4
6
8
10
12
14
16
18
20
22
24
Time (hours)
2 x daily feeding
12 x daily feeding

Fig. II-2. Effect of feeding frequency on acetate/propionate ratio and total VFA concentration. acetate/propionate ratio of cows fed 2-times daily (open circle, broken line), acetate/propionate ratio of cows fed 12-times daily (closed circle, solid line), total VFA concentration of cows fed 2-times daily (open square, broken line), total VFA concentration of cows fed 12-times daily (closed square, solid line).


TOTAL VFA's (mmol/l)
300
200
100
0
ACETATE:PROPIONATE RATIO
3.0
2.0
1.0
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME OF DAY (hours)

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III. EFFECTS OF FEEDING FREQUENCY AND BOVINE SOMATOTROPIN INJECTION ON PERFORMANCE OF DAIRY COWS

A. INTRODUCTION

Dairy cows fed the same quantities of an identical ration can differ markedly in milk production. These differences may be attributed primarily to nutrient partitioning between body stores and milk (3). Therefore an understanding of factors involved in nutrient partitioning can potentially provide opportunities for increasing milk yield in dairy cows. Circulating hormone concentrations, especially somatotropin (BST) and insulin (21), appear to play an important role in nutrient partitioning in dairy cows. It has been demonstrated that high yielding cows have higher plasma BST concentrations and lower plasma insulin concentrations than low producing cows, on a similar dietary regime (18,35). While differences in energy balance may have contributed to observed differences in plasma hormone concentrations, a correlation between plasma BST and insulin concentrations, and milk yield has been demonstrated (20). It has also been shown that BST injections increase milk yield (4,8,24) while milk yield declines in response to insulin injections (32). Thus, maintaining a high BST/insulin ratio would appear to be immportant for maximizing milk yield. While a high plasma BST/insulin ratio may lead to increased milk production, it is not clear how changing concentrations of one hormone influences

the effect of the other. Somatotropin appears to decrease energy uptake by peripheral tissue, and may act to increase mobilization of energy from peripheral tissues, especially adipose tissue, while insulin appears to have the opposite effect (2,6). Therefore, simultaneously increasing plasma BST and decreasing plasma insulin may have an additive effect on milk yield. However, BST may also affect adipose tissue by reducing its responsiveness to insulin (22,36); thus the effect of elevated plasma insulin may be diminished in the presence of high plasma BST. Since high plasma insulin concentration has been implicated as a factor in the low milk fat syndrome (23,36), elevation of plasma BST concentration could reduce milk fat depression by countering the effects of insulin.

In dairy cows it may be possible to manipulate BST by feeding (11,26,33). However, since genetic manipulation of bacteria permits the economical production of large quantities of BST, the most practical method of increasing plasma BST is by direct injection of the hormone. Insulin, on the other hand, may be more easily manipulated by feeding. Increased plasma insulin concentration may result from feeding high concentrate diets (23,31,37) which are neccessary to supply sufficient energy to meet the requirements of high producing cows. Recent work (21) has shown that feeding high concentrate diets more frequently may lower plasma insulin concentrations, especially during the postprandial period. Increasing feeding frequency may counteract the milk fat depressing effects of high concentrate diets.

The objectives of the present study were to examine the interactive effects of BST injection and increased feeding frequency on milk yield, milk composition, and plasma hormone concentrations in Holstein cows.

B. Materials and Methods

Animals, Diet, and Experimental Design

At 45 d postpartum, twelve multiparous Holstein cows were randomly divided into two groups. Six cows were fed concentrate in 12 equal portions at 2 h intervals (12X) using an automatic feeder, and six were fed concentrate in 2 equal portions at 0800 h and 1600 h (2X). All animals received 12.3 kg of concentrate (87.6% DM, 17.3% CP) (Table 1), 2.4 kg of chopped alfalfa hay (81% DM, 21.8% CP) and barley silage (43.6% DM, 9.7% CP) ad libitum. Hay and silage were fed in 2 equal portions at 0900 h and 1500 h. Orts were collected once daily before the morning feeding. Animals were housed in comfort tie stalls and milked in a parlor at 0500 h and 1530 h. At 136 ± 6 d postpartum the cows were used in a single reversal experiment with placebo or BST treatment periods of 8 d. Cows received a daily 10 ml subcutaneous injection of BST[1] (30 mg solubilized as in Peel et al. (27)) or control (solubilization vehicle). Each 8-d injection period was preceded by a 7-d adjustment period. Milk yield was recorded and milk samples collected for 3 d pre-injection, during injection, and for 3 d

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[1]Bovine somatotropin was purchased from A.F. Parlow, Pituitary and Antisera Center, Harbor-UCLA medical Center,1000 W. Carson Stret, Torrance, California, 90509, USA. Lot #8649c, potency 1.9 I.U.

post-injection. Potassium dichromate was added to milk as a preservative and samples were stored at 4°C until analyzed for fat, protein, and lactose. For fatty acid analysis milk samples were collected on each of the last three days of BST or control injection and stored at -20°C until analyzed.

Blood samples were collected hourly for 24 h on the last day of injection, and at 2 and 4 h after the morning feeding for 2 d post-injection. Blood samples were collected via an indwelling jugular catheter inserted on d 7 of injection. Samples were collected in heparinized tubes (12 I.U./ml of blood) containing NaF (15 ul of 4% w/v per ml of blood) and kept on ice until centrifuged at 3000 rpm for 15 min using a Sorvall GCL-2B centrifuge. Plasma was transferred to plastic vials and frozen at -20°C until analyzed.

Analysis

Milk was analyzed for fat, protein, and lactose using infrared analysis[2]. Fatty acid composition of the milk samples was measured using the procedure of (10). Plasma glucose was measured using a Worthington Flozyme kit[3]. Plasma insulin was determined using a double antibody technique. A 100 µl plasma sample or standard was preincubated with guinea-pig antiserum to bovine insulin (200 µl, final dilution 1:165,000) for 24 h at 4°C in 12 x 75

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[2]Milk analysis was done with a Milkoscan 104 manufactured by Foss Electric, Denmark.

[3]Worthington Flozyme Glucose (500nm), catolog No. 37635, Diagnostic Division, Frehold, N.J., 07728, USA.

glass tubes. The following day ^{125}I-labelled insulin (approximately 20,000 CPM in 200 µl) was added and the mixture was incubated for 48 h at 4°C. After incubation the bound hormone was precipitated using 100 µl goat antiserum to guinea pig gamma gobulin (final dilution 1:210), 100 µl normal guinea pig serum[4] (final dilution 1:2100) and a 24 h incubation at 4°C. Tubes were then centrifuged at 3000 rpm for 30 min at 4°C in a Beckman centrifuge, the supernatant aspirated, and the precipitate (bound hormone) was counted using a Beckman gamma 8000 solid scintillation counter. Insulin for standards and iodination was from Sigma[5]. Iodination was carried out using the procedure of Greenwood et al. (17). The dilution buffer (pH 8.6) contained 5.0 g sodium barbital, 2.6 g sodium acetate trihydate, 34.2 ml of .1 N HCl/L and bovine serum added at .5%.

Somatotropin was also measured using a double antibody radioimmunoassay. To 12 x 75 mm glass tubes was added 100 µl of sample or standard, 200 µl rabbit antiserum to BST[6] (1:46,000 final dilution) and 200 µl ^{125}I-labelled BST (approximately 20,000 CPM). This mixture was incubated for 5 d at 4°C. In samples with high concentrations of BST only 50 µl of sample was used with diluent added for constant volume. After a 5-d incubation, 100 µl of goat antiserum

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[4]Antibodies were purchased from Miles Biochemicals, P.O. Box 2000, Elkhart, Indiana, 46515, USA.
[5]Sigma Chemical Co. P.O. Box 14508, St. Louis, Mo. 63178, USA.
[6]Rabbit serrum to bovine growth hormone was generously supplied by Roger Lachance, Centre de Recerches en Endocrinologie Moleculaire Le Centre Hospitalier de l'Universitre lavel 2705, Boulivard Laurier; Quebec G1V 4G2, Canada.

to rabbit gamma gobulin[7] (1:210 final dilution) and 100 µl of normal rabbit serum (1:2100 final dilution) was added and the mixture incubated for 24 h at 4°C. Bound and unbound hormones were separated and counted as described for insulin. Somatotropin used for standards and iodination was obtained from NIH-Lot-B-1. Iodination was according to (17).

The insulin and BST radioimmunoassays were tested for linearity, parallelism and recoveries. All samples were run in one assay to remove inter-assay variation. The intra-assay coefficients of variation were 7.8 and 10.5% for insulin and BST respectively.

C. Results

The effects of BST injection and feeding frequency on milk yield and milk composition are shown in Table 2. Milk yield increased 4.4 kg/d following BST injection ($P<.05$) but was unaffected ($P>.05$) by feeding frequency. Milk yield (Figure 1) increased within 2 d of the initiation of BST injection and reached a plateau after about 5 to 6 d. Following cessation of BST injection, milk production gradually declined with baseline levels being reached after about 4 d. Observed increases in milk production, as well as the temporal pattern of milk yield associated with initiation and cessation of BST injection, were consistent with previous studies (24,27). There were no interactive effects between BST injection and feeding frequency.

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[7]Goat antisera to rabbit gamma gobulin Cat. #539845 was puchased from Calbiochem, Division of Hoerscht Corporation, P.O. Box 12087, San Diego, Ca. 92112, USA.

Milk composition was not significantly affected by BST injection or feeding frequency nor was there an interaction between BST treatment and feeding frequency. Milk fat percent tended ($P<0.1$) to be slightly higher following BST injection. Fatty acid composition of milk (Table 3) was unchanged ($P>.05$) by BST, feeding frequency or interaction between treatment and feeding frequency. There were no differences in the concentrations of individual fatty acids, total long chain fatty acids or short chain fatty acids. Feed intake and body weight (Table 4) were unaffected ($P>.05$) by BST injection, feeding frequency, or interaction between the two. All animals in this study were estimated to be in a positive energy balance with a mean of 9.5 and 4.9 Mcal/d respectively, for control and BST treated animals. Net energy balance was calculated using NRC (1978) values for feed and maintenance while net energy of milk from Tyrrell and Reid (34). Feeding frequency had no effect ($P>.05$) on energy balance.

Average plasma concentrations of insulin, BST, and glucose are shown in Table 5, while 24 h profiles for BST and insulin are shown in Figures 2 and 3 respectively. Feeding frequency had no influence on average or temporal pattern of these parameters, nor was there any interaction of feeding frequency with BST injection. Average plasma BST concentrations were elevated 3-fold by BST injection. Peak concentrations at 4 to 5 h post BST injection were about 7 times control values. Plasma BST levels declined to about 2-times control values at the end of 24 h and were still 1.5-times control values 72 h

after final BST injection. These results are in agreement with previous work (27). Somatotropin injection had no effect on the average or temporal pattern of plasma insulin concentration. Plasma glucose was unaffected by BST injection.

D. Discussion

Somatotropin and insulin are thought to be involved in nutrient partitioning in dairy cows (see reviews 1,12,22). While it has been established that increasing plasma BST by injection will result in increased milk production, the role of feeding frequency and insulin is less clear. Previous work (22) has demonstrated that high plasma insulin concentrations, especially postprandially, may be reduced by increased feeding frequency. As plasma insulin concentration declined, nutrient deposition in adipose tissue appears to be reduced while milk fat percent increased. However this effect was observed in animals fed a 90% concentrate diet where plasma insulin concentration declined from 27.8 μU to 14.4 μU, and milk fat percent increased from 1.79% to 2.97% with increased feeding frequency. In the same study, feeding frequency did not influence plasma insulin concentrations in cows fed a diet containing 70% concentrate even though milk fat percent was increased from 3.26% to 3.92% with increased feeding frequency. In other work (23) animals fed a diet containing 85% concentrates had plasma insulin concentrations which were 50% higher than observed for animals fed a 50% concentrate diet. In contrast, plasma insulin concentration in animals fed a diet containing 75% concentrate was only 25% more than for

animals fed a 50% concentrate diet (37). These results indicate that while plasma insulin concentration may be influenced by concentrate level in the diet, significant effects may only occur at very high levels of concentrate inclusion. In the present study the absence of effect of feeding frequency on plasma insulin, milk yield or milk composition may be a reflection of a lower dietary inclusion level of concentrate than in (22).

Plasma BST concentrations were elevated ($P<.05$) by BST injection resulting in increased yield of milk and milk components. Injection of BST had no influence ($P>.05$) on feed intake, body weight or fatty acid composition of the milk. While body weight and feed intake measurements have limited significance in a short term trial, fatty acid composition of milk can provide an indication of increased fat mobilization from adipose tissue. Fatty acids in milk arise from two sources, de novo synthesis in the mammary gland; which results in a greater percentage of short chain fatty acids, and mobilization of adipose tissue; which results in increased concentrations of long chain fatty acids. The absence of an effect of BST on milk fatty acid composition therefore suggests that the observed increase in milk prodution was not a result of increased intake or fat mobilization but rather a result of an increased diversion of nutrients, from both sources, towards the mammary gland. Increased proportions of long chain fatty acids in milk as a result of BST injection has been atributed to increased mobilization of fat reserves (7). However,

animals in that study were in a negative energy balance in contrast to a positive energy balance in this study. Eppard et. al. (15) observed no change in fatty acid composition of milk when cows were in a positive energy balance, however, as milk yield increased with increasing dose of BST, cows moved into a negative energy balance with a concurrent shift in the proportion of long chain fatty acids in milk. These results suggest that BST injection does not directly increase lipolysis but, rather that lipolysis is affected by energy balance.

One theory of BST action is that it decreases the uptake of nutrients by peripheral tissue, especially adipose tissue, and consequently, more nutrients are "pushed" to the mammary gland. In an in vitro study Vernon (36) found that BST reduced fatty acid synthesis from acetate in adipose tissue even when insulin was included in the medium. Approximately 3 d were required for BST to exert this effect on adipose tissue in vitro. This is consistent with the time interval required for BST injection action on milk yield in vivo. In the present study and other studies (27,29) a minimum of 3 d was required for the effects of BST injection to be fully realized. Thus, it is possible that BST can reduce insulin stimulated removal of glucose and acetate by adipose tissue making these substrates more available to the mammary gland for milk fat and lactose synthesis. This theory is further supported by the fact that glucose oxidation to CO_2 may drop from 34% to 8% with onset of lactation (5). A shift in glucose oxidation at this time coincides with a natural increase in plasma BST.

This possibility could be investigated by feeding a very high concentrates diet which has been shown to increase plasma insulin (22) in conjunction with BST injections.

While BST action may be a result of increased nutrient availability to the mammary gland, other factors must also be considered. In studies using nutrient infusions the magnitude of increases in milk production were much less than with BST injection (22,28) indicating that BST may influence milk synthesis. Somatotropin receptors have not been found in the mammary gland (2). However recent work has shown that insulin-like growth factor-1 (IGF-1) concentration is increased by BST injection (14,30) and IGF-1 may increase milk synthesis by the mammary gland (9). This theory is supported by the observation that BST injection causes an increase in mammary blood flow (13,20). Increased blood flow, probably due to autoregulatory mechanisms, has been associated with increased metabolic activity in many organs (16). If BST were acting on the mammary gland, through IGF-1, to increase milk synthesis then, as production of milk components increased the demand for nutrients would also increase. As feed intake does not immediately respond to BST injection mobilization of body reserves would occur as animals shifted into a negative energy balance. A change in fatty acid composition of the milk should result under these conditions.

In conclusion, at level of concentrates fed on many dairy farms, plasma insulin concentrations may not be influenced by feeding frequency. Injection of BST results in a significant

increase in milk production, although its mechanism of action requires further investigation. On the basis of milk fatty acid composition in the present work and that of (22), BST injection does not appear to have a direct effect on net lipolysis. The extent to which nutrients are "pushed" to the mammary gland as a result of reduced uptake by other tissues or "pulled" to the mammary gland as a result of increased mammary synthetic activity remains to be determined. It seems likely that BST injection acts through a combination of these factors and that as conditions change so does the relative importance of these mechanisms.

Table III-1. Composition of the concentrate.

Ingredient	%, as fed
Barley	71.0
Canola meal	21.5
Molasses	3.0
Calcium carbonate	2.0
Biofos	1.5
Salt	1.0
Vitamin ADE[1]	0.05

[1]Vitamin ADE mixture contained 5000 IU vitamin A, 1280 IU vitamin D_3, and 5 IU vitamin E per kg.

Table III-2. Effects of somatotropin injection and frequency of feeding on milk yield (kg/d) and milk composition.

	Control		Somatotropin		
Feeding frequency[1]	2X	12X	2X	12X	SEM
Milk yield[a]	28.7	27.3	32.4	32.4	0.8
3.5% FCM[a]	27.4	25.2	31.8	31.5	0.8
Milk composition (%)					
Fat	3.76	3.51	3.90	3.82	0.11
Protein	3.21	3.21	3.13	3.21	0.08
Lactose	4.87	4.96	5.13	5.04	0.05

[a]Significant somatotropin effect (P<.05).
[1]Cows fed concentrates twice daily (2X) or 12 times daily (12X)

Table III-3. Effects of somatotropin injection and frequency of feeding on milk fatty acid composition (%).

	Control		Somatotropin		
Feeding frequency[1]	2X	12X	2X	12X	SEM
C 4:0[2]	1.9	1.8	1.6	1.8	<0.1
C 6:0	2.4	2.3	2.1	2.2	<0.1
C 8:0	1.6	1.7	1.5	1.5	<0.1
C10:0	3.8	4.0	3.7	3.5	<0.1
C11:0	0.4	0.4	0.4	0.4	<0.1
C12:0	4.2	4.3	4.1	3.8	<0.1
C14:0	12.0	12.6	12.0	11.4	0.2
C16:0	32.8	33.0	32.5	32.7	0.4
C18:0	7.8	8.0	7.3	8.4	0.2
C18:1	20.6	20.0	22.2	22.2	0.8
C18:2	2.0	2.1	2.0	2.0	<0.1

[1]Cows fed concentrates 2 times daily (2X) or 12 times daily (12X).
[2]Fatty acid length and number of double bonds.

Table III-4. Effects of somatotropin injection and frequency of feeding on DM intake and body weight of cows.

	Control		Somatotropin		
Feeding frequency[1]	2X	12X	2X	12X	SEM
DM intake, kg/d					
Concentrate	11.3	11.3	11.3	11.3	
Chopped hay	2.6	2.6	2.6	2.6	
Barley Silage	10.0	8.9	11.0	9.1	
Total	23.9	22.8	24.9	23.0	0.8
Body Weight, kg	666	645	669	648	23

[1]Cows fed concentrate 2 times daily (2X) or 12 times daily (12X)

Table III-5. Effects of somatotropin injection and frequency of feeding on mean plasma somatotropin (ng/ml), insulin (ng/ml), and glucose concentrations (mg/dl).

	Control		Somatotropin		
Feeding frequency[1]	2X	12X	2X	12X	SEM
Somatotropin[a]	6.28	5.97	19.70	22.75	3.8
Insulin	1.22	1.18	1.76	1.60	0.35
Glucose	59.0	55.3	61.7	62.3	3.5

[a]Significant somatotropin effect ($P<.05$).
[1]Cows fed concentrate 2 times daily (2X) or 12 times daily (12X)

Fig. III-1. Effect of somatotropin (closed square, solid line) or saline (open square, broken line) injection on milk yield.

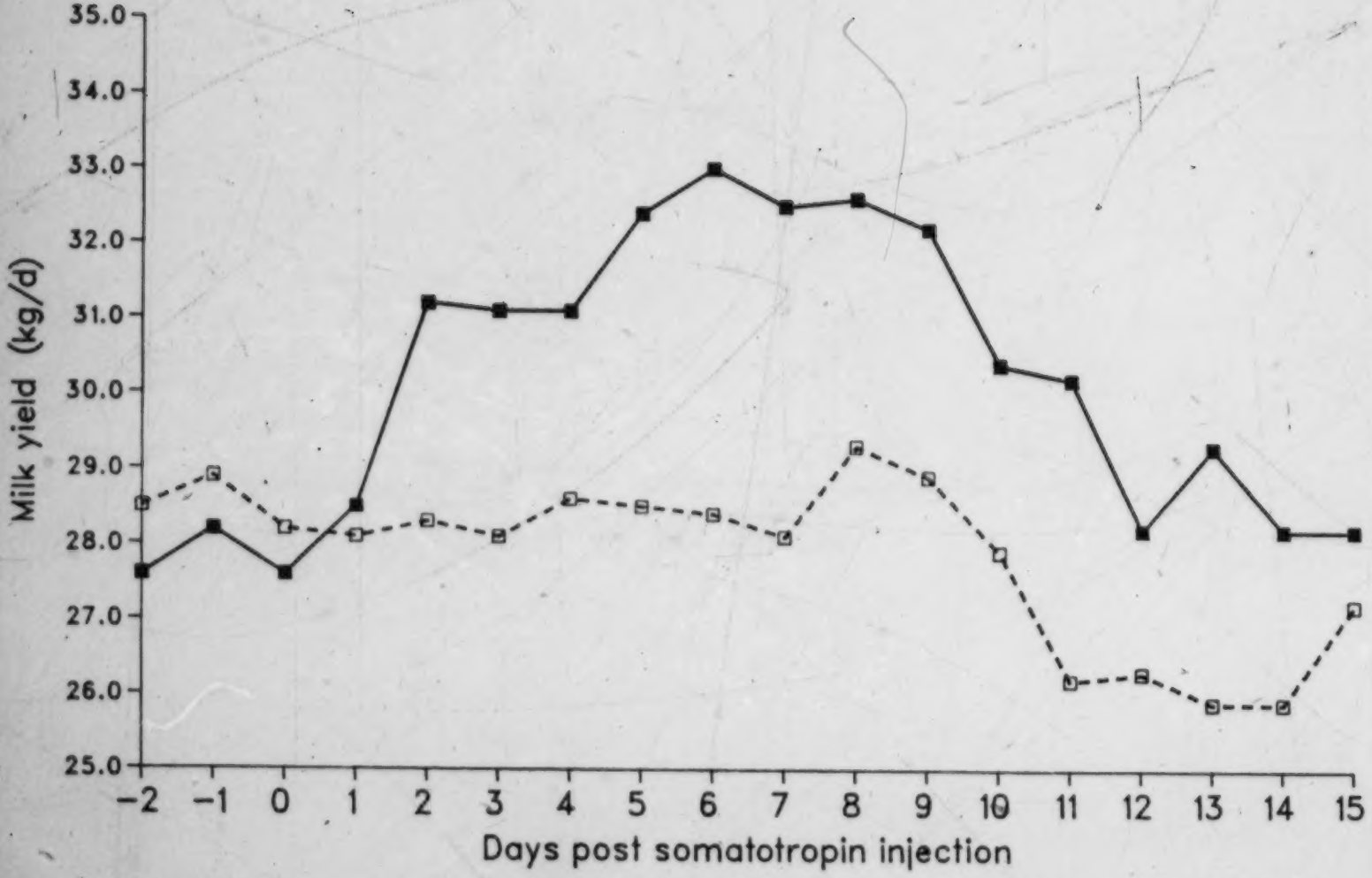

Milk yield (kg/d)
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
27.0
26.0
25.0
−2 −1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days post somatotropin injection

Fig. III-2. Effect of somatotropin or saline injection and feeding frequency on plasma somatotropin concentration; (closed square, solid line) somatotropin injection with 2 times/d feeding; (open square, broken line) somatotropin injection with 12 times/d feeding; (closed circle, solid line) saline injection with 2 times/d feeding; (open circle, broken line) saline injection and 12 times/d feeding.

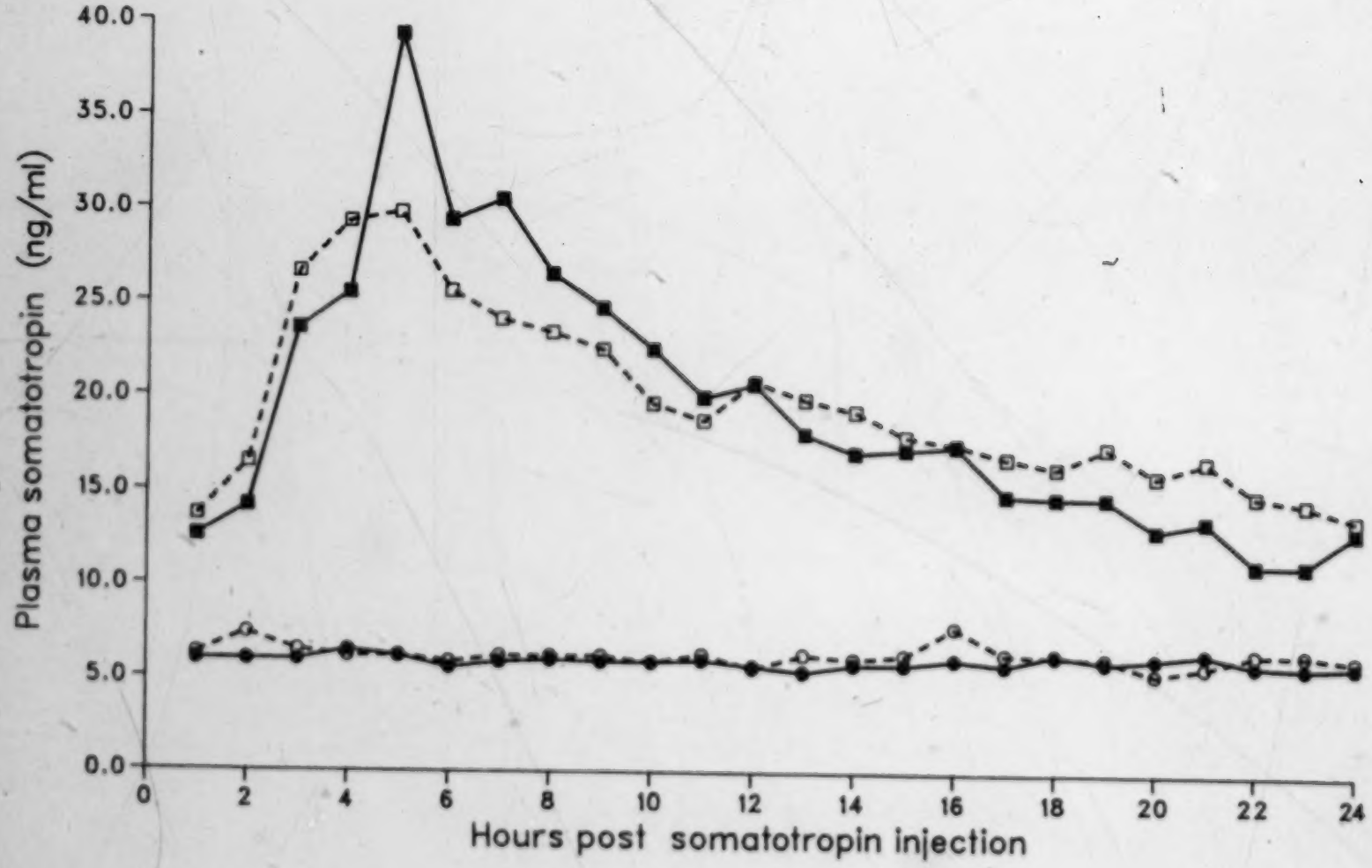
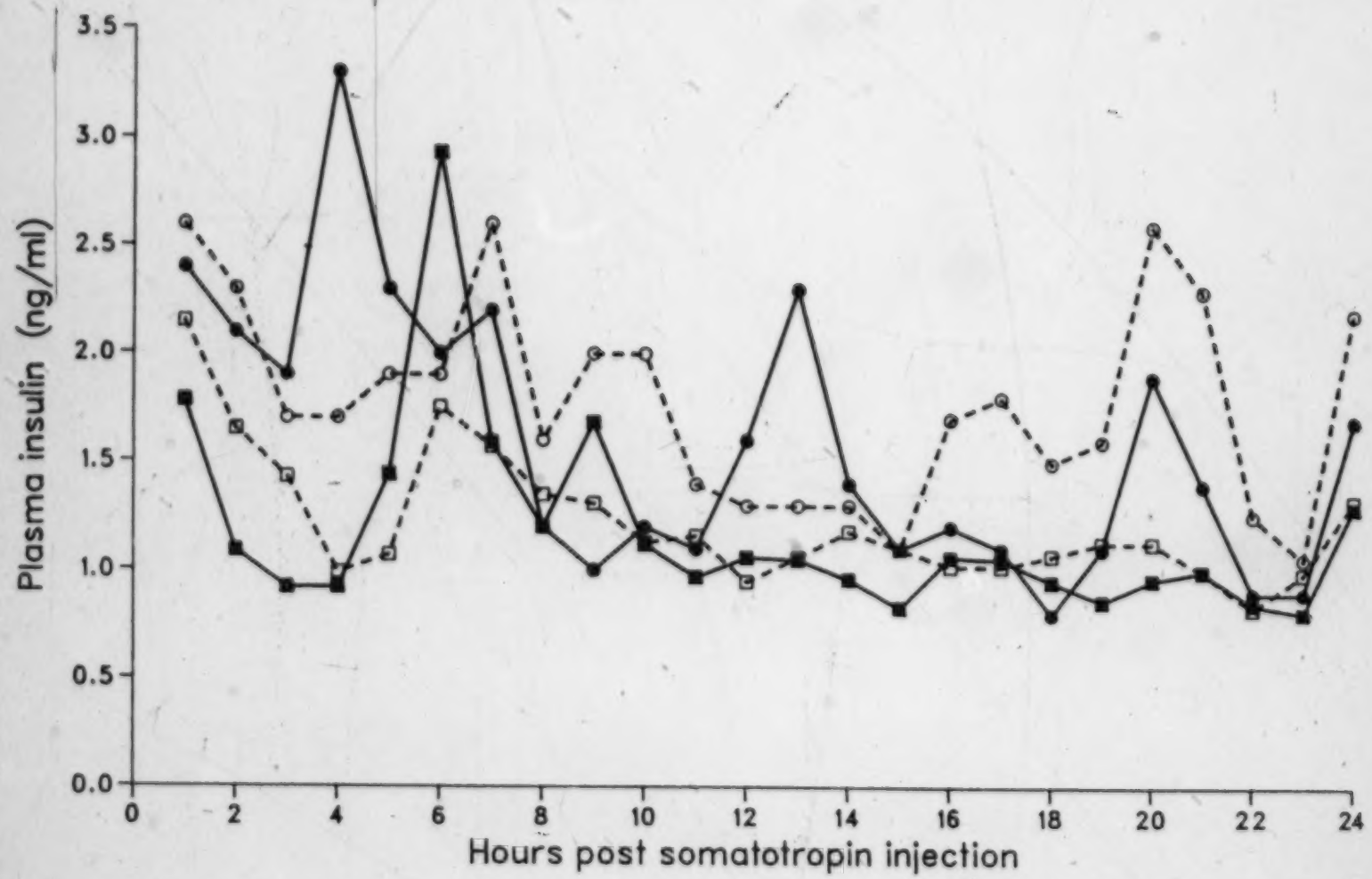

Plasma somatotropin (ng/ml)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
Hours post somatotropin injection

Fig. III-3. Effect of somatotropin or saline injection and feeding frequency on plasma insulin concentrations; (closed square, solid line) somatotropin injection with 2 times/d feeding; (open square, broken line) somatotropin injection with 12 times/d feeding; (closed circle, solid line) saline injection with 2 times/d feeding; (open circle, broken line) saline injection and 12 times/d feeding.


Plasma insulin (ng/ml)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
Hours post somatotropin injection

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IV. General Discussion

Two experiments were conducted to determine the effects of feeding frequency on rumen fermentation characteristics, plasma insulin concentrations, milk yield and milk composition in Holstein cows. In a third experiment the effects of feeding frequency and bovine somatotropin (BST) injection on plasma BST, plasma insulin, milk yield and milk composition in holstein cows were examined.

Feeding frequency did not influence milk yield, milk protein or milk lactose in any of the experiments. In experiment one, where milk fat percent was lowest, increased feeding frequency resulted in increased milk fat percent. However in experiments 2 and 3, where milk fat percent was higher, increased feeding frequency was without effect on milk fat percent. These results are consistent with a recent review by Gibson (9), suggesting that effects of feeding frequency on milk yield, milk protein, and milk lactose are small or nonexistent while the effects of feeding frequency on milk fat percent increase with decreasing milk fat percent. Increased feeding frequency therefore may not influence milk fat percent under many feeding conditions. However, under conditions where milk fat depression is occuring, milk fat percent may increase in response to increased feeding frequency.

The effect of feeding frequency on milk fat percent may be mediated through an effect on rumen pH and acetate:propionate

ratio (14,19). When a diet high in soluble carbohydrates is fed once or twice daily, postprandial VFA production may beelevated resulting in a drop in rumen pH. There is also a shift in fermentation patterns with an increased production of propionate (15). This condition is intensified if the concentrate or the forage is finely ground or pelleted (16). In experiment one, postprandial rumen pH and acetate:propionate ratios were lower in animals fed twice daily as compared to animals fed 12X daily. In experiment 2, rumen pH and acetate:propionate ratio were not influenced ($P>.05$) by feeding frequency. This difference between experiments may be related to the fact that although the diet was identical, animals in experiment 2 were larger and therefore they may have had greater capacity to absorb and/or metabolize VFA's. These experiments support the theory that rumen pH and acetate:propionate ratio influence milk fat percent, and that increased feeding frequency may increase milk fat percent in situations where milk fat depression is occuring.

Another theory of milk fat depression is that high plasma insulin, especially postprandially, promotes storage of acetate, glucose, and free fatty acids by adipose tissue thereby reducing their availability to the mammary gland (11). Elevated plasma insulin concentration can be observed on very high concentrate diets (13,20). This elevation of plasma insulin may be reduced by increased feeding frequency which in turn may increase milk fat percent (11). However, in the

present experiments plasma insulin concentration was not affected by feeding frequency suggesting plasma insulin concentration may not be involved in mediating the effect of feeding frequency on milk fat percent.

In Chapter Two of this thesis, the effects of BST injection on milk yield and milk composition are reported. Somatotropin injection increased milk yield with no change in milk composition. Feed intake and body weights were unchanged, probably due to the short-term nature of the treatments. Fatty acid composition of the milk was also unchanged, and since in general a large proportion of short-chain fatty acids are synthesised from ruminal VFA's while long chain fatty acids are derived primarily from preformed adipose stores; it would seem that increases in milk fat yield were not a result of mobilization of body reserves, or an increase in absorbed VFA's, Rather it appears that BST acts by orchestrating a general shift in partitioning of nutrients towards the mammary gland from both feed and body stores.

Two mechanisms may be involved in this shift in nutrient partitioning. First, nutrient utilization by peripheral tissue could be reduced thus making more nutrients available to the mammary gland. In early lactation when BST concentrations are high, there is less utilization of glucose by peripheral tissue and greater diversion to the mammary gland (1). Also, BST has been shown to reduce insulin stimulated uptake of acetate by adipose tissue (19).While it may be possible to investigate

this theory by elevating the plasma insulin concentrations through feeding high levels of concentrates this effect was not observed at the concentrate inclusion level in diets fed in this study.

A second possible mechanism of BST action may be through it's action on the mammary gland to increase milk synthesis. As milk production increases the demand for nutrients is also increased resulting in an increased partition of nutrients to the mammary gland. It has been suggested that BST has no direct effect on the mammary gland (2) but that it may act by increasing IGF-1 secretion (6,18) which has been demonstrated to affect mammary gland activity (4). It has been demonstrated that mammary blood flow is increased following BST injection (5,10). This supports the theory that mammary activity has been increased by BST injection as blood flow has been shown to increase as a result of increased metabolic activity (8). This may explain why, in some studies, when animals were in a negative energy balance, BST injections increased net lipolysis (3,7) but in experiments where animals are in a positive energy balance, it does not (7 and experiment 3). Also BST does not appear to have lipolytic action in vitro while in vivo it does (12). While BST may affect lipolysis through a second messenger, thus explaining it's in vivo activity, this does not explain why increased net lipolysis occurs only when animals are in a negative energy balance. A more plausable theory is that increased net lipolysis is a result of increased mammary

gland activity causing increased demand by the mammary gland for free fatty acids.

In conclusion, when a rapidly fermented diet is fed, increased feeding frequency may result in higher rumen pH, acetate:propionate ratios and milk fat percent. However the level of concentrate inclusion on many dairy farms may not be high enough to see any effect of feeding frequency. Also, BST injection appears to have potential for increasing milk production under a variety of feeding practices.

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